PATENT
Our Docket: P-TB 4567

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Daniel S. Sem

Serial No.: 09/765,696

Filed: January 19, 2001

For: MULTI-PARTITE LIGANDS AND METHODS OF IDENTIFYING AND USING SAME

Group Art Unit: 1627

Examiner: M. Garcia

#13
3-14-02

Commissioner for Patents
Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Daniel S. Sem, declare as follows:

1) I am the Daniel Sem who is named as the inventor on the above-identified patent application.

2) I understand that the claims of the subject application stand rejected, in part, due to an alleged lack of enablement.

3) I believe that the specification provides sufficient description and guidance to enable one skilled in the art to make and use the invention as claimed.

4) Further in support of the enablement of the claimed invention, described below and in attached Exhibits A through C is the generation of a population of bi-ligands containing at

least one bi-ligand having specificity for a first receptor and at least one bi-ligand having specificity for a second receptor using the teachings in the specification.

5) Briefly, the following is a description of how a population of bi-ligands was generated having specificity for receptors in a receptor family. Computational methods were used to identify common ligands. The coordinates of the nicotinamide mononucleotide portion of the NAD cofactor were used in the gnomonic projection algorithm using well known methods to search databases of commercially available compounds such as ASINEX (Moscow: Russia) and the Available Chemicals Directory (MDL Information Systems, San Diego, CA)(Doucet and Weber, in Computer-Aided Molecular Design: Theory and Applications, Academic Press, San Diego, CA (1996); Bladon, J. Mol. Graph. 7: 130-137 (1989)). Computationally selected common ligands were purchased from ASINEX.

6) The purchased compounds were tested for competitive binding with NADH or NADPH in steady-state kinetic inhibition studies using routine methods (Cleland, Adv. Enzymol. 45:273-387 (1977); Segel, Enzyme Kinetics, John Wiley and Sons, New York (1975); Reddy et al., Biochem. 34:3492-3501 (1995)). One of the compounds identified as a common ligand was structure I shown in Exhibit A. An analog of structure I was prepared where a hydrophobic substituent was replaced with a carboxylic acid (structure II; Exhibit A). This compound was also a competitive inhibitor versus NADH, with K_{is} = 27 mM, and therefore binds in the NADH binding site.

7) NMR experiments were performed with label present in the methyl groups of the Met, Ile or Thr residues obtained by expressing dihydrodipicolinate reductase (DHPR) in minimal media containing labeled Met and Thr using routine methods (Kay and Gardner, Curr. Opin. Struct. Biol. 7:722-731 (1997)). For the NMR experiments, a complex between NADH and DHPR was made, and various perturbations to the cofactor were made using either chemical alterations of the cofactor or radiofrequency irradiations using routine NMR methods. These studies allowed the assignment of amino acid residues of DHPR as being close to the nicotinamide ring of NADH, which is the reactive portion of the cofactor that must be close to the substrate and therefore at the interface between the cofactor and substrate binding sites.

8) The common ligand (compound II of Exhibit A) was bound to DHPR, and routine NMR experiments were performed similar to those performed previously. The catechol ring of compound II was shown to interact with an amino acid distal to the specificity site. To confirm that the portion of the molecule containing the carboxylic acid was proximal to the specificity site and therefore could function as an expansion linker with a reactive functional group oriented towards the specificity site, a propylamide derivative of compound II was generated at the position of the carboxylic acid of compound II, and similar NMR experiments were performed when bound to DHPR. The propylamide derivative of the common ligand (compound III in Exhibit B) showed a clear nuclear Overhauser effect (NOE) between the terminal methyl of the common ligand propylamide derivative and a substrate analog bound to the specificity site, which showed an NOE to an amino acid residue at the interface of the cofactor and

substrate binding sites. Thus, the end of the propylamide group was found to be at an appropriate place for building a bi-ligand library. A carboxylic acid functionality was attached at the end of the propylamide group to facilitate library construction (compound IV in Exhibit B). The positioning of the carboxylic acid functionality oriented to the specificity site, the substrate binding site, was confirmed by NMR (see Exhibit B). Thus, the alkyl group with a carboxylic acid of compounds II or IV can function as an expansion linker to orient a specificity ligand to a specificity site.

9) A bi-ligand library was synthesized with a common ligand and various expansion linkers, including the common ligand and expansion linkers represented by compounds II and IV, using conventional chemical synthesis methods (Parlow et al., J. Org. Chem. 62:5908 (1997)). A library containing about 840 potential specificity ligands and various linkers was generated, resulting in about 3200 compounds, and screened against three enzymes representative of a receptor family that binds NADH/NADPH, DHPR, lactate dehydrogenase (LDH) and 1-deoxy-D-xylulose-5-phosphate reductae (DOXPR), using routine steady-state activity assays measuring competition for binding of NADH or NADPH, as described above. A common ligand and linker bound only weakly to the three enzymes, with K_{is} values in the 25-50 μM range (see Exhibit C; depicted as grey pentagon and arrow showing reactive functionality). Screening the library identified a bi-ligand containing the common ligand of compound IV and a dichlorophenyl group that had a 42 nM K_{is} for LDH (see Exhibit C; bi-ligand with triangle). This represents an increase in potency over the starting common ligand of 1300-fold. This bi-ligand binds 2-3 orders of magnitude stronger to LDH than to DHPR and DOXPR. Another member of the bi-ligand library was identified containing

the common ligand of compound II and a biphenyl ether, which bound with an IC_{50} of 202 nM to DOXPR (see Exhibit C; depicted with circle). This bi-ligand also showed good selectivity, with 60-fold to 120-fold higher affinity for DOXPR than for LDH and DHPR, respectively.

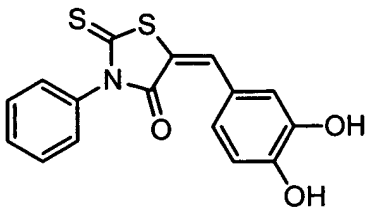
10) In conclusion, the teachings in the specification were used to generate a population of bi-ligands containing at least one bi-ligand having specificity for LDH over DHPR and DOXPR and at least one bi-ligand having specificity for DOXPR over LDH and DHPR. I believe this exemplifies that the guidance provided in the specification is sufficient to show how to make and use a population of bi-ligands.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

2-25-02
DATE

Daniel S. Sem
SIGNATURE

Structure I



Structure II

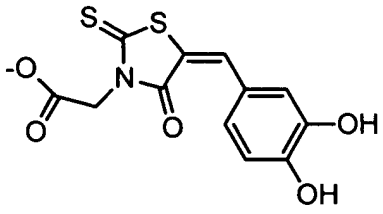


EXHIBIT A

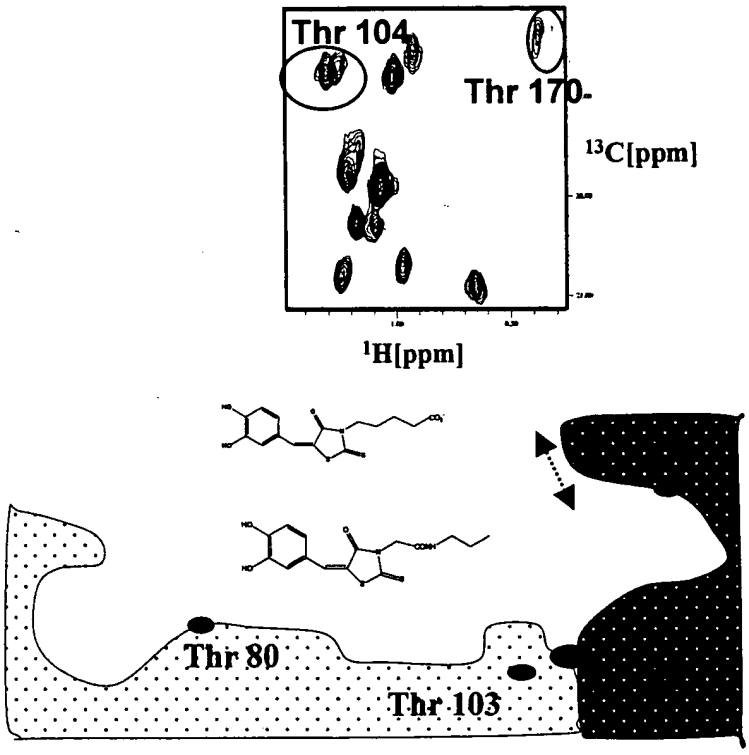


EXHIBIT B

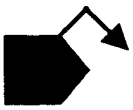
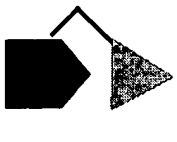
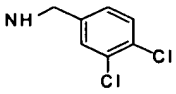
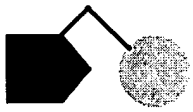
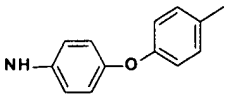
Molecule Type	SL Fragment	LDH	DHPR	DOXPR
	None	55 μM	27 μM	>50 μM
		**42 nM**	>50 μM	10 μM
		12 μM	>25 μM	**202 nM**

EXHIBIT C

Compounds	PKC IC_{50}(μM)	PKA IC_{50}(μM)	Fold affinity of PKC over PKA
5	90	100	1.1
6	8	300	37.5
7	40	50	1.25
8	5	150	30
9	120	310	2.6
10	100	390	3.9
11	60	no inh.	-
12	120	no inh.	-
13	105	390	3.7
14	110	>500	4.5
15	8	210	26.25
16	95	120	1.26

EXHIBIT 2